Status: Preprint has not been submitted for publication

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https://doi.org/10.1590/SciELOPreprints.1734

Submitted on (YYYY-MM-DD): 2021-01-18
Posted on (YYYY-MM-DD): 2021-02-08

"Incidentalormones" - Macro-hormones

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Disclosure of potential conflicts of interest:

None of the authors have any potential conflicts of interest to disclose.

Abstract

Introduction: Complexes of monomeric hormone molecules immunoglobulin-associated lead to the formation of macro-complexes biologically inactive that are called macro-hormones. Patients' presenting unexpectedly elevated hormones values indicates the need that the existence of macro-hormones must be researched.

Objective: To describe the macro-hormones discovered incidentally in laboratory tests, which we refer to as "incidentalormones".

Methods: An integrative review was conducted, and data was gathered from the published articles in medical database. The different forms of macro-hormones are reviewed; the biochemical significance and laboratory assays of macro-hormones are also revised within the ambit of current laboratory medicine. We discussed diagnostic difficulty encountered in patients with "incidentalormones", as well as methods of macro-hormone detection, immunoglobulin involved, clinical significance and associations with other diseases.

Conclusion: The presence of macro-hormones, often guides us to intervention in laboratory trials, and could result in false-positive diagnosis with inadequate therapy. Laboratories should follow a diagnostic algorithm to carefully recognize and examine possible immunoassay interferences.

Key words: Macro-hormones, macro-complexes, inactive hormones, immunoassays.

INTRODUCTION

Complexes of monomeric hormone molecules immunoglobulin-associated or non-immunoglobulin bound lead to the formation of macro-complexes biologically inactive that are called macro-hormones (1). Pitfalls of macro-hormones are rare and unexpected in laboratory examinations, and that is why we refer to it as "incidentalormones".

Macro-hormones are hormone variants with decreased bioactivity in relation to their homologous receptors (2). Because of their high molecular weight, macro-hormones are not effectively eliminated by the renal pathway, circulating normally and being measurable through the available immunoassays despite being biologically inactive.

Although immunoassays have high sensitivity and specificity, various interferences related to the existence of autoantibodies, heterophilic antibodies and even antianimal antibodies can occur, leading to unexpected false-positive and false-negative results (3). Therefore, even with the evolution of immunoassays, analytical errors arising from non-specific reactions or cross reactions are still not entirely preventable.

Hormone levels that are incompatible with the clinical picture are infrequent; however these findings can lead to evaluations by unnecessary propaedeutic methods, false awareness of illness, and inappropriate treatments.

The first report of macro-hormone was made in 1981 by Whittaker et al. when they described a case of hyperprolactinemia with predominance of macroprolactin (4). The term macro-hormone was first used by Jackson et al. in 1985 when he used the term "macroprolactinemia" for a case of very high prolactin levels in a patient with no evidence of pituitary tumor at the imaging examination (5).

Problem solving of analytical measuring or the implications of preanalytic aspects are usually the laboratory's first practice to reply such pitfalls. Laboratories should follow a diagnostic algorithm to carefully recognize and examine possible immunoassay interferences (6). The development of assays less susceptible to antibodies and interfering immunoglobulin are being attempted, however, it has been difficult due to several factors.

In this article we report the different forms of "incidentalormones", their biochemical significance and review the laboratory tests and macro-hormones also in the scope of current laboratory medicine. We discuss the diagnostic difficulty found in patients with "incidentalormones", as well as the prevalence, immunoglobulin involved, clinical significance and associations with other diseases.

METHODS

An integrative review was conducted, and data was gathered from the published articles in various medical databases. The different forms of macro-hormones are reviewed; the biochemical significance and laboratory assays of macro-hormones are also revised within the ambit of current laboratory medicine. We discussed diagnostic difficulty encountered in patients with "incidentalormones", as well as the frequency, immunoglobulins involved, clinical significance and associations with other diseases.

The keywords used produced specific results for documents that used the terms described below.

Laboratory Measurement of Macro-hormones

There are several methods of macro-hormone detection, comprising polyethylene glycol (PEG) precipitation, gel filtration chromatography (GFC), electrophoresis, and ultrafiltration (UF). Although they are different approaches, with limitations and specificities for each method, they have the function of isolating the immunoglobulin-bound hormone of the unbound, or monomeric, form.

- **Polyethylene Glycol precipitation**

PEG precipitation is the most available and most widely used technique to identify macro-hormones. The technique consists of blend same volumetric quantity of PEG reagent and plasma. After centrifuging the mixture, the residual macro-hormone the quantification of the supernatant is performed, where PEG acts reducing the solubility of the proteins leading to its precipitation (7). PEG is fairly essential for precipitation of immunoglobulin complexes and immunoglobulin, and thus precipitates the macro-hormones that contain IgG preferentially while IgA partially precipitates (8). The chance of incorrectly precipitating Ig-complexed analytes with monomeric analytes aggregate also restricts the overview specificity of the PEG reaction, because the plasma can contain high levels of gamma globulin (9).

- **Gel Filtration Chromatography**

GFC is a methodology of separation chromatography utilized to separate molecules of distinct molecular sizes. This technique consists of two phases, a moving phase and a stopped phase due to the relative sizes of the molecules, can be executed with any buffer system that maintains the formation and function of the protein complex (10). Three basic methods are used in the main applications of GFC: desalting or group segmentation, where the evaluated protein and the polluting solutes diverges considerably in molecular size; another method is the application in specification of the molecular size of protein; and an auxiliary protocol to calibrate gel filtration columns for molecular size estimation (11).

- **Electrophoresis**

Electrophoresis is a method that separates immunoglobulin-complexes according to size and electric charge, and which requires more intensive optimization and instrumentation because the preparation of the sample to be analyzed and the laboratory conditions may make it difficult to identify the macro-hormone (12). The electrophoresis is an efficient analytical technique and of great interest for macro-

protein analysis and its use has increased in recent years. The main methods of electrophoresis that separate immunoglobulin complexes from proteins are mainly: gel capillary electrophoresis, micellar electrokinetic capillary chromatography, and the free solution capillary electrophoresis (13).

- **Ultrafiltration**

Newly, it has been demonstrated that the UF technique using exclusive filtration membrane can be useful in the detection of macro-hormones. UF physically withdraws immunoglobulin-complexes according to size. Centrifugation is realized employing a filter with usually 100 kDa porosity that makes possible withdrawal of macro-molecules together with the passage of the monomeric hormone. Research has shown that UF can be an option, particularly in laboratories that have equipment's which use immunoassays that present interference of PEG precipitation (14).

Macro-adrenocorticotropic hormone (macro-ACTH)

Adrenocorticotropic hormone (ACTH) is structurally formed by 49 amino acids residues, being a combination of two regions related to corticotropin-like intermediate lobe peptide and to α-MSH (15). The hypothalamus secretes corticotropin-releasing hormone, which stimulates the release of ACTH that is a tropic hormone secretariat by the anterior pituitary, and that, in turn, acts on the adrenal cortex, controlling cortisol and androgen production by feedback (16).

The ACTH test is often used to diagnose disorders of pituitary and/or adrenal glands, including mainly: Cushing's disease, Cushing's syndrome, Addison disease and hypopituitarism. Thus, the evaluation of plasma ACTH is a very important step for the differential diagnosis of the hypothalamic-hypophysis-adrenal axis dysfunctions.

Electrochemical immunoassays for ACTH dosage are fully automated, with excellent accuracy and a functional sensitivity of 4.8pg/mL, allowing accurate results with wide assay range up to 1000pg/mL, besides presenting low levels of cross-reactivity in an extensive list of potentially interfering substances. The ACTH immunoassay measures the biologically active 39 amino acid chain of ACTH. One biotinylated antibody is prepared to bind only the C-terminal ACTH 34-39 and the other antibody labeled with Horseradish Peroxidase is prepared to bind only the mid-region and N-terminal ACTH 1-24. The reference values are 7.2-63 pg/mL for a.m. draws, and

no reference value has been set for p.m. draws, and these reference values are the same for adults and children (17).

Unexpectedly-high ACTH levels are called macro-ACTH, and are due its complex formation with immunoglobulin. The immunoreactive but bioinactive ACTH molecules are resulting of incomplete cleavage of proopiomelanocortin.

The macro-ACTH is ACTH levels falsely elevated that occur due to interference of anti-analyte antibodies or immunoglobulin-associated. Thus, plasma pretreatment with PEG precipitation or a heterophilic blocking tube substantially reduces its ACTH values. There are only 23 cases of macro-ACTH described in the literature (18).

PEG precipitation of immunoglobulin before to analysis must be utilized to withdraw potentially interfering antibodies. However, state-of-the-art automated analyzers, analysis has become easier and faster. In the PEG precipitation the plasma is mixed with an equal volume of 25% PEG and centrifuged for 5 minutes at 3,000 gravity. The supernatant is measured for ACTH using the specific ACTH assay. Percent of expected value is calculated using the following formula: [(ACTH concentration after treatment) × 2/(ACTH concentration before treatment)] (18). Thus, PEG precipitation study to exclude macro-ACTH should be performed when there is disagreement of the laboratory picture is discordant of the clinical picture.

Macro-Follicle-Stimulating Hormone (macro-FSH)

Follicle-Stimulating Hormone (FSH) is a glycoprotein hormone family, secreted from the anterior pituitary gland that regulates reproduction in mammals. FSH is composed of two different subunits, α and β, where the subunit α being common to glycoprotein hormones, the subunit β specific to FSH, whiles the heterodimer provides its biological activity (19).

The exact laboratory evaluation of FSH levels is fundamental for an accurate diagnosis and successful treatment. Therefore, it is critical that any laboratory measurement of FSH contains the specification of the standard to which the measured FSH was compared and the laboratory method utilized for this comparison.

Macro-FSH should be suspected when FSH levels are discordant with the patient's clinical picture. Thus, hypothesis of macro-FSH should be taken into account in the event of unexplainably elevations of plasma FSH levels (20).

Macro-FSH may be due to the autoimmune response with the presence of anti-FSH antibodies carrying to the formation of an IgG-FSH immunoreactive complex, or

due to the presence of a heterophile antibody or human anti-animal antibody intervening in FSH assay (21).

The serum reference levels of FSH are age-dependent. Currently, the normal limit value of FSH is 20 IU / L for women, below these levels are associated with poor ovarian reserve and reduced fertility, but it is still possible the presence of some ovarian reserve (22). FSH levels far above the appropriate maximum reference range values may be due to the presence of macro-FSH.

Thus, macro-FSH can lead to misdiagnosis, unnecessary evaluation and inappropriate treatment if not distinguished by the relevant laboratory assays. It is essential that laboratories put in their routines a screening to evaluate samples with high levels of total immunoreactive FSH for macro-FSH diagnosis.

Macro-Luteinizing Hormone (macro-LH)

The luteinizing hormone (LH) is a glycoprotein hormone secreted in the adeno-hypophysis, that assist in the maturation of primordial germ cells, the production of testosterone by the Leydig cells of the testicles and the production of estrogens by the ovaries. In addition, LH contributes to the regulation of the menstrual cycle, playing an important role in ovulation and in the implantation of the fecundated egg in the uterus (23, 24).

The LH assay is underpinned on microparticle enzyme immunoassay technology. LH levels vary according to age, sex, clinical history among other variables.

The presence of macro-LH should be suspected when there is a divergence between the clinical picture and the serum level of LH. Macro-LH, as in other macromolecules, is involved with immunoglobulin-G complex and serum autoantibodies (25). Interference of endogenous antibodies in immunoassays of pituitary glycoprotein hormones such as LH is rare (26). Thus, surprisingly high LH values, associated with concomitant FSH and estradiol values within normal reference ranges, should be submitted to macro-hormone research. There are few cases described for LH.

Macro-Thyroid-Stimulating Hormone (macro-TSH)

Thyroid stimulating hormone (TSH) is considered a marker of thyroid function and monitors and directs the treatment of thyroid dysfunctions. TSH is a 28- to 30-kDa glycoprotein secreted in the anterior pituitary gland that leads the thyroid to synthesize

its hormones (T3 and T4). Physiologically, TSH stimulates thyroid growth, protects thyrocytes from apoptosis and plays a fundamental role in ontogeny. Moreover, it participates in the capture of iodine and its organification. The production of TSH in the pituitary gland is dependent on the TSH releasing hormone and blocked by the hormones produced in the thyroid through a feedback mechanism (27).

TSH presents a variation of its serum levels during the day, having higher levels between midnight and 4 a.m. and lower levels in the late afternoon. Thus, a variation in serum TSH levels of up to 50% within the normal range does not strictly represent a change in the functional state of the gland (28). There has been a great development in the sensitivity and specificity of the assays for the measurement of TSH in recent years, changing from a methodology performed by radioimmunoassay with limited functional sensitivity, to immunometric assays with higher sensitivity and specificity (29). The normal reference values of TSH are related to age, in which the elderly have higher concentrations, which do not indicate thyroid dysfunctions, but a natural occurrence of aging (30). The main factors that change in the serum levels of TSH are: biotin, heterophilic antibodies, anti-thyrotropin autoantibodies, anti-ruthenium antibodies, anti-streptavidin, and macro-TSH among others.

Macro-TSH is a macromolecule resulting from the immunoglobulin G and TSH complex, where the serum level of TSH is high and the thyroxin level is normal, simulating a laboratory picture of subclinical hypothyroidism. A recent study showed that 0.79% of individuals with subclinical hypothyroidism had macro-TSH, thus, the distinction between macro-TSH and monomeric TSH in individuals with subclinical hypothyroidism becomes relevant. However, macro-TSH can be observed in individuals with serum levels of TSH within the reference values (31). In gel filtration chromatography the molecular mass of monomeric TSH is 28 kDa while the macro TSH has a molecular mass above 150 kDA (32). The macro-TSH has a low bioactivity and its pathogenesis is not yet fully clarified.

High TSH in routine neonatal screening in a newborn without clinical symptoms may be due to maternal interference, and additional investigations should be carried out, because interfering macro-TSH should be considered in an euthyroid neonate with high serum TSH and normal thyroid hormone levels, thus avoiding unnecessary therapy (33).

Thus, macro-TSH should be considered and researched when isolated serum levels of TSH are incompatible with the patient's clinical picture, especially when significant elevations occur.

Macroprolactin

Prolactin is a 23 kD peptide hormone secreted in the anterior pituitary gland, regulated by the hypothalamus-pituitary-adrenal axis, and physiologically inhibited by dopamine. Prolactin can also be secreted by immune cells, brain, mammary gland, fat tissue, prostate, and ovary. However this extra-pituitary prolactin has different bioactivity and molecular weight (34). The prolactin is fundamental during pregnancy and lactation, because the synthesis and maintenance of milk secretion is its main function.

Biologically there are three isoforms of prolactin: the free monomeric or free little prolactin with a molecular weight of 23 kDa and composed of 199 amino acids and physiologically the most potent, and corresponds to up to 95% of adult serum prolactin; the big prolactin has a molecular weight of 40-60 kDa, a dimer of monomeric form with minimal biological activity; and the big big prolactin or macroprolactin, that is a prolactin-antibody complex with a molecular weight over than 100-150 kDa and represents less than 1% of circulating serum prolactin and also with minimal biological activity (7, 35).

Prolactin serum levels are mainly quantified by the automated immunoassay method. Current immunoassays use a two sites immunometric or sandwich base where prolactin can react with a capture antibody, which is frequently paralyzed in a solid phase, and a characteristic antibody which is used for detection. After the capture of analyte-antibody sandwich, and removal of the reagents that unreacted by a washing step, the signal produced is exactly associated with the amount of current prolactin (36).

Hyperprolactinemia with predominance of macro-molecule of prolactin was the first report of macro-hormone described in 1981 by Whittaker et al (4). The term macro-hormone was first used by Jackson et al. in 1985 when he used the term "macroprolactinemia" for a case of very high prolactin levels in a patient with no evidence of pituitary tumor at the imaging examination (5).

Macroprolactin is the third most frequent cause of non-physiological hyperprolactinemia after prolactinomas, and drug-induced hyperprolactinemia (37). Screening for prolactin and macroprolactin should not be performed in asymptomatic subjects, in order to avoid "incidentalormones", misdiagnosis, and unnecessary treatment. Therefore, an adequate clinical and laboratory evaluation is fundamental for the most adequate therapy.

CONCLUSION

Macro-hormones occur mainly in the form of immunoglobulin IgG complexed hormones. The doctor must be attentive between hormone elevations and disagreement of the clinical characteristics presented by the patient. Thus, the presence of macro-hormones, often guides us to intervention in laboratory trials, and could result in false-positive diagnosis with inadequate therapy. Laboratories should follow a diagnostic algorithm to carefully recognize and examine possible immunoassay interferences.

Conflicts of Interests

None.

Funding

None.

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